Amendments to the Specification under Revised 37 C.F.R. § 1.121

Please amend the specification at page 2, lines 10-21 to read as follows:

The invention provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence as set forth in either SEQ ID NO: 1 or SEQ ID NO: 3;

(b) the nucleotide sequence of the DNA insert in American Type Culture Collection (ATCC) Deposit Nos. PTA-2665 or PTA-2666;

(c) a nucleotide sequence encoding the polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO: 4;

(d) a nucleotide sequence that hybridizes under at least moderately stringent conditions to the complement of the nucleotide sequence of any of (a) - (c); and

(e) a nucleotide sequence complementary to the nucleotide sequence of any of (a) - (c).

Please amend the specification at page 27, lines 7-17 to read as follows:

Preferred methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are described in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux *et al.*, 1984, *Nucleic Acids Res.* 12:387; Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA (Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-10). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (Altschul *et al.*, *BLAST Manual* (NCB National Library Medicine NIH, Bethesda, MD); Altschul *et al.*, 1990, *supra*). The well-known Smith Waterman algorithm may also be used to determine identity.

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(312) 913-0001

Please amend the specification at page 33, lines 9-30 to read as follows:

Where all or only a portion of the flanking sequence is known, it may be obtained using PCR and/or by screening a genomic library with a suitable oligonucleotide and/or flanking sequence fragment from the same or another species. Where the flanking sequence is not known, a fragment of DNA containing a flanking sequence may be isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes. Isolation may be accomplished by restriction endonuclease digestion to produce the proper DNA fragment followed by isolation using agarose gel purification, ~~Qiagen®~~-column chromatography (QIAGEN Inc., Chatsworth, CA), or other methods known to the skilled artisan. The selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

An origin of replication is typically a part of those prokaryotic expression vectors purchased commercially, and the origin aids in the amplification of the vector in a host cell. Amplification of the vector to a certain copy number can, in some cases, be important for the optimal expression of a TGF-β-R polypeptide. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector. For example, the origin of replication from the plasmid pBR322 (New England Biolabs, Inc., Beverly, MA) is suitable for most gram-negative bacteria and various origins (*e.g.*, SV40, polyoma, adenovirus, vesicular stomatitus virus (VSV), or papillomaviruses such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (for example, the SV40 origin is often used only because it contains the early promoter).

Please amend the specification at page 39, lines 12-25 to read as follows:

Preferred vectors for practicing this invention are those that are compatible with bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3, and pcDNA3.1 (Invitrogen Corp., San Diego, CA), pBSII (Stratagene Corp., La Jolla, CA), pET15 (Novagen, Inc.,

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Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech Laboratories, Inc., Palo Alto, CA), pETL (BlueBacII, Invitrogen Corp.), pDSR-alpha (International Pub. No. WO 90/14363) and pFastBacDual (~~Gibco~~ GIBCO-BRL, Grand Island, NY).

Additional suitable vectors include, but are not limited to, cosmids, plasmids, or modified viruses, but it will be appreciated that the vector system must be compatible with the selected host cell. Such vectors include, but are not limited to plasmids such as Bluescript® plasmid derivatives (a high copy number ColE1-based phagemid; Stratagene ~~Cloning Systems~~ Corp., La Jolla, CA), PCR cloning plasmids designed for cloning Taq-amplified PCR products (*e.g.*, TOPO[[™]] TA Cloning® Kit and PCR2.1[[®]] plasmid derivatives; Invitrogen Corp.), and mammalian, yeast or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives; Clontech Laboratories, Inc.).

Please amend the specification at page 41, lines 6-10 to read as follows:

Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described, for example, in Kitts *et al.*, 1993, *Biotechniques*, 14:810-17; Lucklow, 1993, *Curr. Opin. Biotechnol.* 4:564-72; and Lucklow *et al.*, 1993, *J. Virol.*, 67:4566-79. Preferred insect cells are Sf-9 and Hi5 (Invitrogen Corp.).

Please amend the specification at page 43, lines 21-31 to read as follows:

The purification of a TGF–β–R polypeptide from solution can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as Hexahistidine (TGF–β–R polypeptide/hexaHis) or other small peptide such as FLAG (Eastman Kodak Co., New Haven, CT) or *myc* (Invitrogen Corp.) at either its carboxyl- or amino-terminus, it may be purified in a one-step process by passing the solution through an affinity column where the column matrix has a high affinity for the tag.

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For example, polyhistidine binds with great affinity and specificity to nickel. Thus, an affinity column of nickel (such as the ~~Qiagen~~®-nickel columns of QIAGEN Inc.) can be used for purification of TGF–β–R polypeptide/polyHis. *See, e.g., Current Protocols in Molecular Biology* § 10.11.8 (Ausubel *et al.*, eds., Green Publishers Inc. and Wiley and Sons 1993).

Please amend the specification at page 57, lines 6-17 to read as follows:

Another *in vitro* assay that is useful for identifying a test molecule that increases or decreases the formation of a complex between a TGF–β–R polypeptide binding protein and a TGF–β–R polypeptide binding partner is a surface plasmon resonance detector system such as the BIAcore® assay system (Pharmacia AB Corp., Piscataway, NJ). The BIAcore® system is utilized as specified by the manufacturer. This assay essentially involves the covalent binding of either TGF–β–R polypeptide or a TGF–β–R polypeptide binding partner to a dextran-coated sensor chip that is located in a detector. The test compound and the other complementary protein can then be injected, either simultaneously or sequentially, into the chamber containing the sensor chip. The amount of complementary protein that binds can be assessed based on the change in molecular mass that is physically associated with the dextran-coated side of the sensor chip, with the change in molecular mass being measured by the detector system.

Please amend the specification at page 78, lines 17-20 to read as follows:

Deposits of cDNA encoding isoforms 1 and 2 of human TGF–β–R polypeptide, subcloned into the pGEM®-T Eeasy vector (Promega Corp.; Madison, WI), having Accession Nos. PTA-2665 or PTA-2666, were made with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209 on November 10, 2000.

Please amend the specification at page 79, line 11 to page 80, line 15 to read as follows:

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PCR amplifications were then performed using 50 ng of cDNA as a template (obtained from one of 77 proprietary human tissue cDNA libraries), 5 *p*mol each of a suitable 5' and 3' amplimer, and Ready-To-Go® PCR Beads (Amersham Pharmacia Biotech AB Corp., Piscataway, NJ), according to the manufacturer's recommended procedure. Reactions were performed at 94°C for 1 minute for one cycle; 94°C for 15 seconds, 62°C for 30 seconds, and 72°C for 1 minute for 40 cycles; and 72°C for 7 minute for 1 cycle. A 339 bp PCR product was obtained in amplification reactions containing the amplimers 2445-29 and 2445-32 and either a fetal ovary or fetal skin cDNA library template.

To isolate full-length cDNA sequences for the human TGF–β–R polypeptide, 5'- and 3'-RACE was performed using the Advantage-High Fidelity 2 PCR kit (Clontech Laboratories, Inc.), 50 ng of either the fetal ovary or fetal skin cDNA libraries, and a "touchdown" PCR protocol (Don *et al.*, 1991, *Nucleic Acids Res.* 19:4008). The 5'-RACE reactions were performed using 10 *p*mol of the amplimers 2445-32 and 1916-83 (5'-G-G-C-T-C-G-T-A-T-G-T-T-G-T-G-T-G-G-A-A-T-T-G-T-G-A-G-C-G-3'; SEQ ID NO: 24), the latter amplimer corresponding to the nucleic acid sequence of the vector used to construct the cDNA libraries (pSPORT). The 3'-RACE reactions were performed using 10 *p*mol of the amplimers 2445-29 and 1916-80 (5'-T-G-C-A-A-G-G-C-G-A-T-T-A-A-G-T-T-G-G-G-T-A-A-C-G-C-C-A-G-3'; SEQ ID NO: 25), the latter amplimer corresponding to the nucleic acid sequence of pSPORT. Reactions were performed at 94°C for 2 minutes for one cycle; 94°C for 5 seconds and 72°C for 4 minutes for 5 cycles; 94°C for 5 seconds and 69°C for 4 minutes for 5 cycles; 94°C for 5 seconds and 67°C for 4 minutes for 25 cycles; and 72°C for 7 minutes for 1 cycle.

Following 5'- and 3'-RACE, nested PCR was performed using the Advantage-High Fidelity 2 PCR kit, 10 μl of a 1:50 dilution of the first round 5'- or 3'-RACE amplification products, and an appropriate pair of amplimers, in a volume of 50 μl. For amplification of the 5'-RACE product, the amplimers 2450-22 and 1916-82 (5'-C-A-T-G-A-T-T-A-C-G-C-C-A-A-G-C-T-C-T-A-A-T-A-C-G-A-C-T-C-3'; SEQ ID NO: 26) were used. For amplification of the 3'-RACE product, the amplimers 2450-21 and 1916-81 (5'-T-C-A-C-G-A-C-G-T-T-G-T-A-A-A-A-C-G-A-C-G-G-C-C-A-G-T-G-3'; SEQ ID NO: 27) were used. Reactions were performed at 94°C for 2 minutes for one cycle; 94°C for 5 seconds and 72°C for 4 minutes for 5 cycles; 94°C for 5 seconds and 70°C for 4 minutes for 5

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Chicago, Illinois 60606
(312) 913-0001

cycles; 94°C for 5 seconds and 68°C for 4 minutes for 25 cycles; and 72°C for 7 minutes for 1 cycle. Following separation on a 1% agarose gel, well-defined PCR products were isolated from the gel, purified using a gel extraction kit (~~Qiagen~~ QIAGEN Inc.), and then sequenced.

Please amend the specification at page 81, lines 11-19 to read as follows:

Multiple human tissue Northern blots (Clontech Laboratories, Inc.) were hybridized with a 540 bp probe generated by PCR amplification of human TGF-β-R cDNA using the amplimers 2445-28 and 2445-31. The probe was radioactively labeled using a ~~Redi-Prime~~ Rediprime II kit (Amersham Biosciences; Piscataway, NJ) according to the manufacturer's instructions. Northern blots were prehybridized for 30 minutes at 60°C in ~~Rapid-Hyb~~ Rapid-hyb buffer (Amersham Biosciences), and then hybridized in a hybridization oven (Stratagene Corp.) for 1 hour at 60°C in ~~Rapid-Hyb~~ Rapid-hyb buffer containing the radioactively labeled probe. Following hybridization, the blots were washed twice in 2X SSC and 0.1% SDS at room temperature and then once in 0.1X SSC and 0.5% SDS for 1 hour at 68°C. Hybridized blots were examined by autoradiography.

Please amend the specification at page 82, lines 4-15 to read as follows:

Following hybridization, sections are rinsed in hybridization solution, treated with RNaseA to digest unhybridized probe, and then washed in 0.1X SSC at 55°C for 30 minutes. Sections are then immersed in NTB-2 emulsion (Eastman Kodak Co., Rochester, NY), exposed for 3 weeks at 4°C, developed, and counterstained with hematoxylin and eosin. Tissue morphology and hybridization signal are simultaneously analyzed by darkfield and standard illumination for brain (one sagittal and two coronal sections), gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, proximal colon, and distal colon), pituitary, liver, lung, heart, spleen, thymus, lymph nodes, kidney, adrenal, bladder, pancreas, salivary gland, male and female reproductive organs (ovary, oviduct, and uterus in the female; and testis, epididymus, prostate, seminal vesicle, and vas deferens in the male), BAT and WAT (subcutaneous, peri-renal), bone (femur), skin, breast, and skeletal muscle.

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Please amend the specification at page 82, lines 19-29 to read as follows:

PCR is used to amplify template DNA sequences encoding a TGF–β–R polypeptide using primers corresponding to the 5' and 3' ends of the sequence. The amplified DNA products may be modified to contain restriction enzyme sites to allow for insertion into expression vectors. PCR products are gel purified and inserted into expression vectors using standard recombinant DNA methodology. An exemplary expression vector, pCEP4 (Invitrogen Corp., Carlsbad, CA), that contains an Epstein-Barr virus origin of replication, may be used for the expression of TGF–β–R polypeptides in 293-EBNA-1 cells. Amplified and gel purified PCR products are ligated into pCEP4 vector and introduced into 293-EBNA cells by lipofection. The transfected cells are selected in 100 μg/mL hygromycin and the resulting drug-resistant cultures are grown to confluence. The cells are then cultured in serum-free media for 72 hours. The conditioned media is removed and TGF–β–R polypeptide expression is analyzed by SDS-PAGE.

Please amend the specification at page 83, lines 5-12 to read as follows:

Inclusion bodies containing TGF–β–R polypeptide are purified as follows. Bacterial cells are pelleted by centrifugation and resuspended in water. The cell suspension is lysed by sonication and pelleted by centrifugation at 195,000 x*g* for 5 to 10 minutes. The supernatant is discarded, and the pellet is washed and transferred to a homogenizer. The pellet is homogenized in 5 mL of a Percoll® solution (75% liquid Percoll® and 0.15 M NaCl) until uniformly suspended and then diluted and centrifuged at 21,600 x*g* for 30 minutes. Gradient fractions containing the inclusion bodies are recovered and pooled. The isolated inclusion bodies are analyzed by SDS-PAGE.

Please amend the specification at page 86, line 20 to page 87, line 2 to read as follows:

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At 8 weeks of age, transgenic founder animals and control animals are sacrificed for necropsy and pathological analysis. Portions of spleen are removed and total cellular RNA isolated from the spleens using the Total RNA Extraction Kit (~~Qiagen~~ QIAGEN Inc.) and transgene expression determined by RT-PCR. RNA recovered from spleens is converted to cDNA using the SuperScript[[™]]® Preamplification System (~~Gibco~~ GIBCO-BRL) as follows. A suitable primer, located in the expression vector sequence and 3' to the TGF–β–R polypeptide transgene, is used to prime cDNA synthesis from the transgene transcripts. Ten mg of total spleen RNA from transgenic founders and controls is incubated with 1 mM of primer for 10 minutes at 70°C and placed on ice. The reaction is then supplemented with 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM of each dNTP, 0.1 mM DTT, and 200 U of SuperScript® II reverse transcriptase. Following incubation for 50 minutes at 42°C, the reaction is stopped by heating for 15 minutes at 72°C and digested with 2U of RNase H for 20 minutes at 37°C. Samples are then amplified by PCR using primers specific for TGF–β–R polypeptide.

Please amend the specification at page 87, line 16 to page 88, line 4 to read as follows:

The spleen, lymph node, and Peyer's patches of both the transgenic and the control mice are subjected to immunohistology analysis with B cell and T cell specific antibodies as follows. The formalin fixed paraffin embedded sections are deparaffinized and hydrated in deionized water. The sections are quenched with 3% hydrogen peroxide, blocked with Protein Block (Lipshaw Co., Pittsburgh, PA), and incubated in rat monoclonal anti-mouse B220 and CD3 (Harlan Bioproducts, Indianapolis, IN). Antibody binding is detected by biotinylated rabbit anti-rat immunoglobulins and peroxidase conjugated streptavidin (BioGenex Laboratories Corp., San Ramon, CA) with DAB as a chromagen (BioTek Solutions, Inc., Santa Barbara, CA). Sections are counterstained with hematoxylin.

After necropsy, MLN and sections of spleen and thymus from transgenic animals and control littermates are removed. Single cell suspensions are prepared by gently grinding the tissues with the flat end of a syringe against the bottom of a 100 mm nylon cell strainer (Becton, Dickinson and Co., Franklin Lakes, NJ). Cells are washed twice, counted, and approximately 1 x 10^6 cells from each

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tissue are then incubated for 10 minutes with 0.5 μg CD16/32(FcγIII/II) Fc block in a 20 μL volume. Samples are then stained for 30 minutes at 2-8°C in a 100 μL volume of PBS (lacking Ca^+ and Mg^+), 0.1% bovine serum albumin, and 0.01% sodium azide with 0.5 μg antibody of FITC or PE-conjugated monoclonal antibodies against CD90.2 (Thy-1.2), CD45R (B220), CD11b (Mac-1), Gr-1, CD4, or CD8 (PharMingen Corp., San Diego, CA). Following antibody binding, the cells are washed and then analyzed by flow cytometry on a FACScan (Becton, Dickinson and Co.).

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300 South Wacker Drive
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(312) 913-0001